R0-modeling as a tool for early warning and surveillance of exotic vector borne diseases in Denmark

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Published in:
Disease vectors today: Changes in ecology, climate and public health risks

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

Disease vectors today:
Changes in ecology, climate and public health risks

Kipi-Koovi Hiking Centre, Saaremaa, Estonia

2011, May 24-27th

ABSTRACT BOOK

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SMITTSKYDDSINSTITUTET

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Baltic Sea Unit

Organizers:

Nordic Viral Zoonoses Network

Nordic-Baltic Network for Tick-borne Diseases

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DNA barcodes as a tool for identification of biting midges, potential vectors of bluetongue virus

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Background: Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are the insect vector of economically important veterinary diseases such as African horse sickness virus, Epizootic hemorrhagic disease virus and bluetongue virus (BTV). Bluetongue is caused by a double stranded RNA virus and infects ruminants. Transmitters of the BTV in Europe are *Culicoides imicola* and potential vector species *Culicoides obsoletus*, *Culicoides scoticus*, *Culicoides dewulfi*, *Culicoides chiopterus* and *Culicoides pulicaris*. However, identification of these based on morphological features are difficult. The use of DNA barcodes has been proposed as a tool for rapid identification to species for many groups of insects. Hence, a study was undertaken to construct DNA barcodes for all morphologically determined species in the Swedish collections.

Methods: In order to construct DNA barcodes for species collected in Sweden, mitochondrial cytochrome oxidase subunit I (COI) was chosen as marker. Specimens were sequenced from a small piece of tissue and the head, wings and genitalia were mounted and determined to species according to relevant keys. All sequences were compared against data available in Genbank.

Results: In total, 235 specimens of *Culicoides* morphologically identified to be 37 species were used. DNA barcode approach based on sequences of COI gene could identify 35 species among these. However, two pairs of closely related species, *Culicoides festivipennis* and *Culicoides clastrieri* could not be separated based on DNA barcode approach as well as *Culicoides manchuriensis* and *Culicoides salinarius*. Out of 35 species identified with barcodes nine were available in Genbank for comparison, of these eight matched well. However, specimens of *Culicoides lupicaris* did not cluster together with available sequences, though this species is known to be versatile. Each potential vector species possessed distinctive sets of COI haplotypes which discriminated well among species. However, *C. obsoletus* showed one specimen which had high divergence from other specimens. Furthermore, two species, *Culicoides newstedi* and *Culicoides halophilus* are listed as synonyms but showed high sequence divergence which indicates that they are separate species.

Conclusions: The use of COI barcodes as a tool for species identification of biting midges can differentiate 90% of species studied, for some closely related species a less conserved region such as ribosomal ITS spacer needs to be employed.

Entomological studies of blood-sucking dipteran insects (Diptera) as a basis for future vector studies of haemosporidian parasites (Haemosporida) in the Baltic region

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Bloodsucking dipteran insects (Diptera) are vectors of haemosporidian parasites (Haemosporida), which cause diseases in birds and other vertebrates all over the word, including the Baltic region. Vector species, which transmit certain haemosporidian species remain insufficiently investigated. We discuss the available data on four groups of potential avian haemosporidian vectors from Lithuania, Latvia and Curonian spit (Russia, Kaliningrad).

Mosquitoes (Diptera: Culicidae) are specific vectors of *Plasmodium* spp. Thirty-seven mosquito species are known in this region with *Ochlerotatus communis* and *Ochlerotatus cataphylla* predominating from the middle of May till the end of June, and *Aedes vexans* and *Culex pipiens* predominating from July till September. Adult mosquitoes are active till the end of October each year depending on the air temperature.

Black flies (Diptera: Simuliidae) are specific *Leucocytozoon* spp. vectors. Twenty-seven species of black flies are known in the region, but only 10 of them were recorded as bloodsuckers. Bloodsucking black flies are active in May – June. *Simulium maculatum* and *Simulium reptans* dominate among bloodsucking black flies in Lithuania, *Simulium ornatum* is the main pest species in Latvia. No cases of bloodsucking black flies were registered in Curonian spit due to the lack of running waters (rivers, streams, etc.) at this study site.

Biting midges (Diptera: Ceratopogonidae, Culicoides) are vectors of *Haemoproteus* and *Leucocytozoon* spp. Twenty-seven biting midge species are reported, with *Culicoides obsoletus* group predominating from the middle April, *Culicoides impunctatus* and *Culicoides punctatus* predominating from the beginning of June. Biting midges of *Culicoides obsoletus* group are active till the end of October. Predominant species of *Culicoides* can vary and depend on the habitat. Louse flies (Diptera: Hippoboscidae) are also vectors of several *Haemoproteus* spp. Eight species are known in Lithuania but only 3 of them are ornithophagous, 4 ornithophagous species of louse flies are known from Latvia and from the Curonian spit.

In the Baltic region, vectors of haemosporidians have been subjects of research only on the Curonian spit (Kaliningrad, Russia). Particular attention was paid to the relationship between vectors and avian haemosporidians, which are actively transmitted in Europe. To determine which species of haemosporidians can be transmitted by certain species vectors, biting midges were experimentally infected with avian *Haemoproteus* spp. The formation of sporozoites in salivary glands of vector and subsequent infection of recipient uninfected birds with sporozoites was used to identify haemoproteid vectors. *Culicoides impunctatus* was shown to be the competent vector for 5 species of avian haemoproteids. Importantly, haemoproteid infection was highly virulent for these insects, with high mortality among experimentally infected midges.

Further studies are needed to determine species of blood-sucking insects, which are responsible for transmission of haemosporidian parasites in wildlife. Entomological studies indicate directions to plan such research.

Climate change and fish parasites

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A warmer sea is likely to result in a different parasite exposure. We are testing this by analysing MHC-profiles in the perch from the Biotest lake outside Forsmark nuclear power plant. The Biotest Lake has been enclosed from 1977 to 2007 and has a water temperature that is 6-10 degrees warmer than the surrounding waters all year round. We are using data from 1977 and onwards to 2009 both within the Biotest Lake and from a control population outside the enclosure. In this way we can get an understanding of the temporal change of MHC-allele frequencies as a result of living in warmer water and thus get an understanding of the change in parasite pressure over time.

R_0-modeling as a tool for early warning and surveillance of exotic vector borne diseases in Denmark

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Modeling the potential transmission intensity of insect borne diseases with climate driven R0 process models is frequently used to assess the potential for veterinary and human infections to become established in non endemic areas. Models are often based on mean temperatures of an arbitrary time period e.g. a monthly temperature mean. Temperature decreases with latitude, and in the Nordic countries periods of suitable temperatures, the windows of opportunity for transmission, may be very short and only appear in odd years. While average monthly temperatures are likely to be suitable for predicting permanent establishment of presently exotic diseases, mean temperatures may not predict the true potential for local spread and limited outbreaks resulting from accidental introductions in years with temporary periods of warm weather.

DTU-Veterinary Institute is developing a system for continuous risk assessment of potential local spread of exotic insect borne diseases of veterinary and human importance. R0 models for various vector borne diseases are continuously updated with spatial temperature data to quantify the present risk of autochthonous cases ($R_0>0$) and the present risk of epidemics ($R_0>1$) in case an infected vector or host are introduced to the area.

The continuously updated risk assessment maps functions as an early warning system allowing authorities and industry to increase awareness and preventive measures when R_0 raises above the level of 'no possible transmission' and target active serological surveillance to these limited periods of potential risk, thus dramatically reducing the number of samples collected and analysed. The risk estimated from the R_0 modelling may be combined with the risk of introduction from neighboring countries and trading partners to generate a truly risk based surveillance system for insect borne diseases.

R_0 models for many vector borne diseases are simple and the available estimates of model parameters like vector densities and survival rates may be uncertain. The quantitative value of R_0 estimated from such models is therefore likely to deviate from the true R_0. However assuming the models are qualitatively able to rank the estimated R_0 correctly, a period resulting in a relatively high estimated R_0 will also be a period with a relatively high true R_0. This allows the estimated R_0 to be used for targeted surveillance by focusing the surveillance on periods and areas with high R_0 estimates even if the actual value of these estimates are difficult to interpret. Furthermore running R_0 models on historic outbreaks in Europe may be used to fit estimates for R0 for these data. When comparing the model R_0 to the observed value of R_0 a correction factor is obtained that may be used to adjust the model estimates in Denmark, and thus allowing a more quantitative interpretation of the estimated R_0. This presentation will demonstrate the system for selected vector borne diseases and compare the predicted R_0 with the actual spread of bluetongue in Scandinavia in 2008.

Assessing the Effects of Climate and ENSO Variability on Dengue Fever Incidence in 23 Mexican Provinces

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Our aims were three-fold: First, we examined the associations of dengue incidence with climate and El Niño variability in 23 Mexican provinces. Second, we assessed how much these associations varied among provinces. Finally, we explored climate-, geography- and development-related sources of variability for these associations. We fitted negative binomial regression models and use their results to conduct a series of meta-analysis. Data were collected for the period January 1985 to December 2007. Our results demonstrate that DF incidence is associated with climate and El Niño variability in 91% of the studied provinces. The strength and type of association with the climatic predictors were highly heterogeneous. This heterogeneity was not due to random. Using meta-regression models we demonstrated that at least part of the heterogeneity observed in the associations with climate and El Niño variability can be attributed to the underlying dominant climate of each province and their proximity to El Niño region 3.4. The influence of the underlying dominant climate as a moderator was corroborated in direct and indirect manners. None of the studied associations appeared to be influenced by development-related moderators. This observation demonstrates that associations of DF incidence with climate and El Niño variability are independent from development. This suggests that climate change is likely to worsen dengue incidence in the region by increasing temperatures in the country as well as changing its geographical distribution. It also stresses the need for tailored policies and control measures for each province.

Recycling of animal by-products – recycling of pathogens?

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The use of animal by-products, ABP, (e.g. manure, slaughter house waste, catering waste) as fertilizer and soil conditioner in agriculture will close the loop of plant nutrient recycling and contribute to a sustainable society. However, arable use of ABP can unintentionally spread infectious disesases. The health risks associated with recycling of ABP are bacteria (e.g. verotoxin producing *E. coli*. (VTEC), *Salmonella* spp.), virus (e.g. classical swine fever, foot and mouth disease) and parasites (e.g. *Toxoplasma* spp, *Ascaris* spp.). Many of these pathogens are zoonoses and have the ability to pass between humans and animals.

Large-scale livestock production, epizootic diseases, introduction of new pathogens as a result of the climate change and increasing globalisation increase the need for biosecurity and the need to minimise the risk of disease transmission in the food chain. On farms dissemination of pathogen can occur via animals or the handling and spreading of manure and other kinds of ABP, and further via surface runoff to water and via harvested crops. New diseases can also be introduced via transport of animal by-products between nations. Thus, to obtain a general acceptance for arable use of ABP, a hygienically safe end-product is needed. Several different treatments such as composting, anaerobic digestion and ammonia treatment can be used to obtain such end-products.

Inactivation of pathogens during treatments is influenced by several different parameters such as temperature, time, available nutrients, pH and moisture content. Furthermore the inactivation rate depends on the species since different microorganisms are more or less sensitive to external conditions. Inactivation of pathogens will also occur over time in the environment due to sunlight, drought, high temperatures etc. However, this kind of inactivation is unpredictable and should not be overestimated. The risk for spread of infections can also be decreased through the use of barriers such as restriction on grazing after spread of ABP.

Borrelia species in Estonian tick populations

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Lyme borreliosis is the most frequent tick-borne disease in Europe as well as in Estonia. There are two tick species circulating in Estonia – *Ixodes ricinus* which is spread throughout the whole territory of a country, and *Ixodes persulcatus* prevalent in the East part of the country. Both tick species are vectors for Borrelia sp. spirochetes. According to the data from Estonian Health Board, there were 1721 cases of tick borreliosis in 2010, that is 128.4 cases per 100 000 population. The highest morbidity was on Saaremaa island – 1342.0 cases per 100 000 population (466 cases). In this study we analyzed ticks collected in 2006- 2009 in different parts of Estonia mainland and on Saaremaa islands for the presence of Borrelia sp. Another part of a study was detection of Borrelia genotypes. Borrelia sp. detection was done using PCR for 16S rRNA gene and/or by reverse-line blot hybridization assays (RLB). Genetic characterization of *Borrelia burgdorferi sensu lato* complex strains was performed with PCR for 5S-23S intergenic spacer region. For genetic characterization of *Borrelia miyamotoi* p66 and glpQ genes were chosen. Totally, 1980 individual ticks were analysed (104 nymphs and 268 adults of *I.persulcatus* and 526 nymphs and 1082 adults of *I.ricinus*). Borrelia sp. was detected overall in 189 ticks. The most frequently detected was *B.afzelii* (25.8%), followed by *B.garnii* -9.6%, *B.miyamotoi*-6.6%, *B.valaisiana* -5% of Borrelia-positive ticks. *B. burgdorferi sensu stricto* and *B.lusitaniae* was detected in 0.5% (1 sample) each. 13.1% of Borrelia-positive samples had co-infection with several Borrelia genotypes. 34.3% of positive samples have not been genotyped yet.

The structure and assembly of Hantaviruses

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Hantaviruses are rodent- and insectivore-borne members of the family Bunyaviridae. The hantavirus infection of the host causes presumably a life-long persistence, throughout which the host secretes the virus in its excreta (feces, urine and saliva). If transmitted to man, hantaviruses may cause either hemorrhagic fever with renal syndrome (HFRS, in Eurasia) and hantavirus cardiopulmonary syndrome (HCPS, in the Americas).

The negative-sense RNA genome of hantaviruses is segmented into three segments S, M and L that respectively encode for nucleocapsid (N), glycoproteins Gn and Gc, and RNAdependent RNApolymerase (RdRp). The genome segments are encapsidated by N protein to form RNP that is enclosed inside a lipid envelope decorated by spikes generated by Gn and Gc. The formed virion is approximately 70 to 210 nm in diameter and displays round or pleomorphic morphology.

The focus of this study was to understand the mechanisms and interactions through which the virion is formed and maintained. We started by analyzing the interactions between the spike-forming glycoproteins Gn and Gc and observed them to favor homo- over hetero-oligomerization (Gn-Gn and Gc-Gc contact is stronger than Gn-Gc). Based on interaction data we created a model, wherein tetrameric Gn complexes are interconnected by homodimeric Gc units to form the grid-like surface architecture described for hantaviruses.

Structural study using electron cryo-tomography resulted in a three dimensional (3D) reconstruction of the spike complex at 3.6 nm resolution. The spike complex was 10 nm high, and displayed a four-fold symmetry with dimensions of 15 nm times 15 nm. The structural data agreed with the biochemical characterization of the glycoprotein interactions, thus suggesting the four-fold symmetry to be maintained by Gn-Gn interactions.

The cryo-tomographic data suggested an interaction between the cytoplasmic tail (CT) of Gn and RNP. We were able to verify this interaction using monoclonal antibodies and synthetic peptides. Further characterization indicated that both Gn and Gc are likely to contribute to the interaction between the spike complex and RNP. These results led us to suggest a model where after successive formation of the spike complex, the Gn-CT becomes available for interaction with RNP, and this drives the budding of virions at the site of assembly.

Large discrepancy between the current estimated incidence of Lyme borreliosis and the number of diagnoses made in primary care in Sweden

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The current Swedish incidence estimate for Lyme borreliosis is 69/100 000 based on data from southern Sweden, where the disease was temporarily made notifiable during 1992-93. Most borrelia infections are diagnosed in primary care and treated with antibiotics. Diagnoses are usually made on clinical symptoms only, as laboratory tests are not reliable in early illness. There is no national registry collecting information on primary care diagnoses, but many counties keep computerised registries for internal use. We attempted to acquire incidence data from these registries in order to get updated data on the incidence of Lyme borreliosis in Sweden.

We contacted all Swedish counties and asked for primary care data on number of individuals diagnosed with Lyme borreliosis (ICD code A69.2) in 2008.

Five out of 21 counties could provide complete datasets. Nine could provide data from some primary care units and seven could provide very little or no data. The 2008 incidence per 100 000 for counties that could provide complete data was 421 in Kronoberg, 529 in Södermanland, 530 in Västra Götaland, 390 in Örebro and 476 in Östergötland.

Complete national data could not be obtained. The average incidence of primary care Lyme borreliosis diagnoses in 2008, in counties that could report, was 6.8 times higher than the current Swedish estimate. The difference may signify an increase, large geographical or yearly variations. Alternatively the incidence based on diagnoses may be falsely high. There is a need to investigate this discrepancy further. If it is due to false diagnoses it would imply that many patients are given unnecessary treatments, which is bad both from a patient and an antibiotic resistance perspective.

Monitoring of bloodsucking insects in Lapland (Finland)

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Several early explorers of Lapland described the horror of the bloodsucking insects. Olaus Magnus, who in the 15th century were selling letters of indulgence in the North, wrote that sleeping was impossible unless you had a small tent of linen. It was not only their sting but also their insufferable sound. Pitch-oil was the best repellent. According Johannes Schefferus the reindeers had to be moved to the high mountains during summer because of the insects.

The abundance of mosquitoes, gnats and biting midges during the short subarctic summer is called "räkkä" in Finnish and everyone who has visited Lapland in summer time is familiar with it. Only a few studies have been done on the ecology of the phenomena. The species composition and the phenology are largely unknown. Most efforts have been done to investigate the impact of the larger bloodsucking dipterans.

The on-going project started in 2010. The preliminary monitoring started with one trap (MosquitoMagnet) in May and continued to October when the permanent snow cover was formed. The monitoring was done at the Reindeer Research Station in Kaamanen, Inari (Lapland). During two weeks in July one trap was also kept at Svanvik in the Pasvik Valley (Norway).

Only a small part of the insect material has been processed and only a few species has been determined. The most common Mosquito species in June was *Anopheles messeae*. The adult females hibernate and start looking for blood meals for egg laying. In July there was no *Anopheles*. The first gnats also appeared before midsummer. The main top of mosquitoes and gnats started in the end of June. In August the number of both mosquitoes and gnats decreased. The main top of biting midges was in August – September. They were flying until the occurrence of the permanent snow cover.

Malaria eradication, household size and climate

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During the 20th century deliberate attempts to eradicate malaria commenced in many countries. Eradication succeeded in some parts of the world but failed in other parts. Malaria also disappeared spontaneously in several countries. The exact reason has remained an enigma. Early malariologists saw a connection between malaria and poverty. With the introduction of DDT the impact of the social variables were forgotten and replaced by overconfidence in mosquito control.

High frequency trend of malaria was explained by meteorological data which affected the annual variations of the vector populations. The low frequency trend, which will lead to malaria eradication, was affected by social variables. Data on malaria, vectors, demographic factors, sociological factors, and environmental factors from 232 countries were compiled. In the final analysis 189 countries with presence of malaria and vectors or eradicated malaria were used. Correlation analysis and multivariate analysis of malaria frequency and various parameters were performed. Timing of disappearance of malaria in relation to declining household size was analysed.

GDP per capita, household size, female literacy and urbanization are significantly correlated with malaria frequency. Household size showed a distinct lower threshold value of four members when malaria disappeared. This threshold value was globally valid across all climate zones irrespective of counter measures, vector species, or *Plasmodium* species.

The significance of household size can be explained by the behaviour of the vector. Transmission of malaria is mostly an indoor event. When the vector becomes infective it gets increased biting rate and therefore the number of people catching malaria depends on the household size. Long range dispersal is done by humans but short range dispersal is made by both humans and mosquitoes. In big households the human share of local dispersal and prevalence of malaria is larger than in smaller households.

Development of Microarrays in the Diagnosis of Viral Hemorrhagic Fevers

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Viral hemorrhagic fever (VHF) is an acute infection with a high mortality rate. RNA viruses belonging to families *Filoviridae*, *Bunyaviridae*, *Flaviviridae*, and *Arenaviridae* can be causative agents of VHF. These viruses have similarities such as a single-stranded RNA genome and lipid envelope.

Rapid diagnosis of hemorrhagic fever viruses is of importance due to the severity of the illnesses. Travelers can be infected while visiting endemic areas and vectors carrying the virus can be transmitted by worldwide freight and therefore non-endemic countries have to be prepared for diagnosis of these viruses.

Methods used for laboratory diagnosis of VHFs include isolation of the virus in cell culture or laboratory animals, polymerase chain reaction (PCR), virus antigen detection, electron microscopy, and detection of specific antibodies in the patient serum.

Microarray method is based on multiplex-RT-PCR and is used for detection of multiple virus targets at the same time. This is beneficial for diagnosis of wide variety of viruses causing same type of illness, e.g. VHFs. Therefore a microarray method for diagnosis of VHFs was developed.

Multiplex-RT-PCRs for detection of Ebola, Marburg, Dengue 1-4, Yellow Fever, Crimean-Congo Hemorrhagic Fever, Rift Valley Fever, Lassa and LCM viruses were developed and microarray for identifying PCR products was set up. Evaluation of the method is ongoing.

Ixodes persulcatus carries TBEV-Eur in Finnish Lapland

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Finland is at the northernmost edge of tick-borne encephalitis (TBE) endemic area in Europe. Recently, human cases occurred at the northernmost coastal area of the Baltic Sea (in Simo, 65°40'N, 24°54'E), approximately 100 km south from the Arctic Circle.

Both tick species *Ixodes ricinus* and *I. persulcatus* are found in Finland but their distribution areas seem not to overlap. Instead, *I. ricinus* is found in southern and central parts of the country while, notably, we have found *I. persulcatus* in scattered foci along the western coast, including Kokkola archipelago, where *I. persulcatus* carries Siberian subtype of the TBE virus (TBEV).

In June 2009 we collected 97 *I. persulcatus* ticks and 17 *Myodes glareolus* (bank voles) from Simo at places where TBE patients had likely contracted a tick bite. We pooled the ticks to 51 pools of 1-3 ticks; the rodents were handled individually. For virus isolation experiments we chose the two TBEV-RNA positive tick pools (of two and three ticks) as well as four rodents which had detectable levels of antibodies to TBEV by IFA and TBEV RNA in lung and brains. We isolated 6 (4 from bank voles, 2 from *I. persulcatus)* TBEV-Eur strains.

Simo in Finnish Lapland is a new and by far the northernmost TBE endemic focus known in the world. The tick species in Simo is *I. persulcatus* (the taiga tick). However, the isolates represented the TBEV-Eur subtype, unlike other strains usually reported from *I. persulcatus,* suggesting that the virus and the tick vectors have had different dispersal routes and histories.

Tick-borne pathogens in Estonia: their prevalence and genetic characterization

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Tick-borne pathogens (TBP) are widely distributed, and play an important role in human morbidity all over the world. Tick-borne diseases (TBD) are infectious zoonotic diseases occurring in endemic areas or natural foci where the circulation of TBP is maintained over long periods of time. Tick-borne encephalitis virus (TBEV) is a well studied TPB and the disease caused by it, tick-borne encephalitis (TBE), is widely distributed and well recognized in Europe and in Estonia. There is also an increasing number of cases of human granulocytic anaplasmosis (HGA) and human babesiosis reported in Europe; diseases caused by *Anaplasma phagocytophilum* and *Babesia* species, respectively. The *Ixodes* ticks are the main vectors of TBP in Europe. There are two tick species of the *Ixodes* genus circulating in Estonia: *I. ricinus,* prevalent throughout the entire territory, and *I. persulcatus,* found in Eastern Estonia. The co-circulation of two *Ixodes* species is unique for Europe, where only *I. ricinus* circulates, and therefore studies of the presence, circulation and genetic characteristics of TBP in Estonia are important and significant.

In the present study, an analysis of specific amino acid substitutions in the E glycoprotein of the Estonian TBEV strains belonging to the Siberian subtype was carried out. A study of the circulation as well as genetic characterization of the zoonotic tick-borne pathogens *Anaplasma phagocytophilum* and *Babesia* species *B. microti*, *B. divergens*-like and *Babesia* sp. EU1, were performed in Estonia for the first time.

The first goal of this study was to analyze strains of the TBEV- Siberian subtype circulating in Estonia. Phylogenetic analyses showed that Estonian strains and strains from Latvia and Finland form a well supported Baltic lineage within the TBEV-Sib subtype. We found a new unique signature amino acid substitution within the Siberian subtype of TBEV at position 175 for Baltic strains and 313 for Siberian strains, and could thus discriminate the Baltic TBEV-Sib strains from strains isolated in Siberia and the Far-East. Furthermore, protein structure modelling and geometrical analysis of the E protein surface showed, that both amino acids are located close to each other and to the receptor-binding site and may influence the receptor binding properties of the virus.

The second goal of this study was to investigate the presence and prevalence of *A. phagocytophilum* in Estonian ticks and to provide a genetic characterization and phylogenetic analysis of local *A. phagocytophilum* strains. The present study showed that *A. phagocytophilum* was detected only in *I. ricinus* ticks from all investigated sites with prevalence rates varying from 1.7 to 2.6%. Genetic analysis demonstrated that the Estonian strains of *A. phagocytophilum* belonged to different *groESL* lineages, and represented different variants of 16S rRNA and the *ankA* genes. We suggest that ticks may harbor all repertoires of *A. phagocytophilum* that are circulating in the same area, while genetic variants of *A. phagocytophilum* segregate in specific natural hosts. Thereby ticks may be an optimal source for studies on the diversity of *A. phagocytophilum* in various geographical regions.

The last goal of this study was to analyze *Babesia* species circulating in Estonia. In the present study, the circulation of three Babesia species, *B. microti*, *B. divergens*-like and *Babesia* sp. EU1, was detected, with prevalence rates of up to 1.4% in Estonian ticks. *B. microti* was detected in both tick species, while *Babesia* sp. EU1 and *B. divergens*-like were only found in *I. ricinus* and *I. persulcatus*, respectively. Genetic studies based on the analysis of partial 18S rRNA gene revealed that Estonian sequences of *Babesia* species shared a high rate of similarity and were closely related to *B. microti*, *B. divergens*-like and *Babesia* sp. EU1 strains from other European countries, Siberia and the USA. Moreover, the analyzed sequences in the 18S partial gene region of *B. microti*, *B. divergens*-like and *Babesia* sp. EU1 were identical to sequences reported in infected patients; however, human infections due to *Babesia* species have to date not been reported in Estonia.

A comparison of microscopy and PCR diagnostics for low intensity infections of blood parasites in birds

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This study compares the sensitivity of a polymerase chain reaction (PCR)-based method and microscopy examination of blood smears for diagnosing avian malaria (*Plasmodium* and *Haemaproteus*) and closely related hematozoan parasite, *Leucocytozoon*, prevalence in Siberian tits (*Poecile cinctus*) breeding in northern Finland. During molecular analysis we used PCR screening and RFLP as post-PCR diagnostic step. The blood parasites were found in the blood of 95% of 40 breeding Siberian tits by using molecular methods, while the prevalence of avian malaria and *Leucocytozoon* spp. was found to be significantly lower when the same samples were tested by microscopy. However, habitat and sex effects on the total count of parasites, *Plasmodium* spp, *Haemaproteus* spp. and *Leucocytozoon* spp. counts appeared to be similar when obtained by either screening methods. Microscopy examination of blood smears and PCR diagnostics showed the same prevalences for *Lecocytozoon* spp. infections. Prevalences of *Haemaproteus* spp. and Plasmodium spp. determined by molecular methods were significantly higher than the prevalence determined by microscopy screening. The results of this study suggest that PCR diagnostic and subsequent use of RFLP analysis may be sufficient to avoid the risk of co-amplification of closely related genera of blood parasites, while microscopy is a reliable method for detecting blood parasites of birds except for diagnosing chronic, low intensity infections.

Host-virus interactions of tick-borne encephalitis virus

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Host innate immune responses against flavivirus infections are mediated by different leukocyte types. Natural killer (NK) cells, granulocytes, macrophages and dendritic cells are responsible for the induction of proinflammatory and antiviral responses. Type I interferons (IFN-α/β) play a critical role in this response. The complement system is also part of innate immunity consisting of small proteins circulating as inactive proenzymes. Some large DNA viruses use as much as 50% of their genome to produce proteins that interfere with the host immune system. Smaller viruses, such as flaviviruses, have restrictions in genome size, so they have had to evolve genes with multiple functions. This study is focusing on the effects of individual TBEV proteins on induction of type I interferons and the activation of the complement system.

Searching for causative agents of fever of unknown origin in Finnish travelers to tropics - ongoing project

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This presentation will discuss an ongoing project, which aims to determine a microbiological etiology for Finnish patients with travel history to tropics, and who subsequently present with an unknown origin of fever. The primary approach is to serologically identify acute viral infections in such patients. The first subset of patients screened for this study has been a cohort of Finnish travellers who have been suspected of Dengue virus infection in 2004-2009, and of whom paired sera are available. For this cohort, we have began the investigation with Chikungunya alfavirus and hantavirus antibody screening. The preliminary results of the first subset of patients will be presented.

Generation of reporter Tick-borne encephalitis virus-like particles

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Subgenomic replicons of positive-stranded RNA viruses contain all of the genetic elements needed to amplify themselves in susceptible host cells but lack some or all of the genes coding for structural proteins. Consequently, these RNAs are replicated in cells but are not packaged into viral particles. Replicons have proven to be valuable tools for studying replication independently of virion assembly and maturation. Moreover, they have great potential as molecular tools for gene expression and as vectors for therapeutic and prophylactic purposes. The delivery of replicon RNA to host cells can be achieved by encapsidation of replicon RNA with viral structural proteins provided in *trans*. Such virus-like particles (VLPs) are capable of initiating a single round of infection, providing an efficient and easy way to deliver the replicon vector into specific host cells. If these VLPs are engineered to express a reporter such as the green fluorescent protein, they may serve as tools in vaccine testing and drug development efforts.

The aim of this work is generation of reporter tick-borne encephalitis virus-like particles and evaluation of their biological properties.

Earlier the tick-borne encephalitis virus (TBEV) replicon was constructed in which the structural genes of TBEV have been deleted, leaving the cyclization sequences in the 5' end of C plus sequences encoding for the C-terminal (~25 amino acid residues) of E, to serve as the secretory signal for the immediately downstream NS1 gene. After this a cassette containing the encephalomyocarditis virus internal ribosome entry site (IRES) fused to the GFP-encoding gene was cloned into the replicon between NS5 and the 3' NTR. This bicistronic design will allow the NS proteins to be translated and processed as it would happen in virus-infected cells.

In order to encapsidate the replicon RNA of TBEV we have constructed the recombinant plasmid pSFV-PG+C-prM-E Kumlinge A52, encoding structural proteins of TBEV, using noncytopathic Semliki Forest virus vector. To prepare the VLPs, BHK21 cells will be first transfected with the *in vitro* transcribed replicon RNA, followed 8 h later by a second transfection with RNA SFV-PG+C-prM-E Kumlinge A52. Following the second transfection, the culture supernatant will be harvested and used to infect naive cells. At 48 h post infection, the cells will be monitored for GFP fluorescence and evidence of cytopathic effects. The generated VLPs could be applied to neutralization testing for the detection of specific antibodies against TBEV.

Abundance of Japanese Encephalitis Virus vectors in South Vietnam – association with urban agriculture

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Japanese Encephalitis Virus (JEV) is a vector-borne Flavivirus, causing disease in large parts of South and East Asia. The virus is spread by mosquitoes between wild birds, pigs and humans. Whereas wild birds are free of symptoms and pigs only show signs of disease if they are pregnant, when the virus cause abortions and deliver stillborn fetuses, humans can develop encephalitis with high case fatality rates and severe sequelae for many survivors. Pigs are considered amplifying hosts.

In many countries where the virus is present there are concomitant problems with poverty and increasing urbanization. To secure their food supply many poor urban inhabitants keep smaller livestock such as pigs and poultry close to their homes.

Here we study 12 households with and 5 without pigs in a city in south of Vietnam, where JEV occurs endemically. Mosquitoes were caught using CDC mini light traps during night, killed by freezing and identified. The numbers of the three mosquito vector species most common in the catches were analyzed in relation to the number of people and pigs in the households, presence of other livestock, rice fields or fishponds and the overall density of humans and animals in the municipality.

The total number of mosquitoes, as well as the number of *Culex tritaeniorhynchus*, increased with increasing numbers of pigs in the household ($p= 0.004$ and 0.02 respectively) and in the catches closest to the pigs ($p<0.0001$ and $p=0.0004$ respectively). There was however no association between *Culex quinquefasciatus* and the number of pigs at all, but this species was positively associated with the number of people in the household ($p= 0.03$) and negatively associated with the density of large ruminants ($p=0.004$). This mosquito is known to be an anthropophilic species. *Culex gelidus* showed a complex association with several parameters on the farm but again there was more mosquitoes caught close to the pigs ($p<0.0001$).
This study show that 3 wellknown vectors are present within an urban society and that 2 of the species show an increase in number with increasing number of pigs kept in the urban agriculture.

Reconstruction of ancestral virions, a genetic approach

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It is known that enteroviruses frequently recombine within species but rarely between species. Recombination may occur throughout the genome but is highly restricted within the P1 region coding for the structural proteins. Typing of enteroviruses are now performed by analyses of sequence identity within the P1 region where identity of 75% or more of the VP1 part (the most variable structural gene) at the nucleotide level corresponds to the same type and are also correlated to viruses of the same serotype. Molecular epidemiology analyses of coxsackievirus B5 (CVB5) isolates demonstrate two monophyletic groups of co-circulating isolates. The Human Enterovirus B (HEV-B) species consists of group B coxsackieviruses, echoviruses, coxsackievirus A9 and recently identified enterovirus types. We have constructed a cassette-vector for HEV-B using a replication backbone of a laboratory strain of CVB5 and a complete P1 region that can be replaced by replacement cloning. Complete sequence analyses of eight P1 regions (structural genes) of currently circulating CVB5 isolates enabled us to predict the ancestor P1 (P1anc) region of the two co-existing groups of circulating CVB5. In silico synthesis and cloning of the P1anc into the cassette vector followed by transfection of the full-length cDNA clone resulted in viable and replicating virions of a putative CVB5 ancestor, CVB5-P1anc. The features of replicating CVB5-P1anc and circulating CVB5 clinical isolates were compared and will be presented.

Rodent-borne viruses

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Several of the most dangerous viruses, causing highly lethal diseases in humans, have rodents as natural reservoirs. The majority of these important human pathogens are found in the Bunya- and Arenavirus families. The lecture will give an overview of the rodent-borne viruses, their rodent hosts and the diseases they cause in man.

Parasitic manipulation of appetite in Yellow-Necked Mouse

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One common physiological phenomenon that is involved both in infectious and malignant processes is the reduction in appetite – disease anorexia. Potential candidates for inducing disease anorexia include various neuro-endocrine products that are known to regulate appetite in healthy animals including humans. These include leptin, ghrelin, cholecystokinin, insulin and somatostatin. Leptin a 16 kDa cytokine that has complex physiological effects including appetite regulation and is similar to other cytokines associated with the inflammatory response. An increase in plasma levels of leptin, both when associated with inflammation and when occurring in healthy organisms, has been shown to suppress appetite in several kinds of vertebrates. We wanted to test whether a parasitic infection with *Cysticerus fasciolaris* the larvae of the heleminth parasite *Taenia taeniaformis* would affect the levels of appetite regulating proteins such as leptin, gherlin, and neuropeptide Y (NPY). From an evolutionary perspective it would be more adaptive for an organism that is internally parasite within its host and that is constantly growing to stimulate the appetite of the host instead of causing its suppression. Infections with *C. fasciolaris* are rather common in wild Yellow-necked mouse (*Apodemus flavicollis*). The infection is thought to be asymptomatic and is considered harmless, however the eco-physiological effect of the infection is not well known. In the present experiment we investigated the effect of an internal parasite infection with *C. fasciolaris* on the plasma levels of appetite regulating neuro-endocrine substances leptin, ghrelin and neuropeptide Y (NPY) in wild Yellow-necked mice. We found that infected mice had significantly lower plasma levels of leptin and increased levels of NPY compared to the healthy subjects. Ghrelin levels were not associated with the occurrence of the parasites, however, these levels strongly correlated with the levels of NPY. This study suggests a possible manipulation by the parasitic larvae of the appetite regulation in infected subjects.

The Arms Race of Vector Borne Diseases: the evolutionary dance continues

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The issues surrounding the taming of both human and animal vector borne diseases are usually multifactorial and complex. To help us understand the relationships between the players at present we can benefit by understanding how past changes including spatial, ecological and temporal considerations have led to shape the evolutionary milieu. Nowhere is this more apparent than the results of economic globalisation that began with human migrations in the last thousand years. This has led many former sheltered ecologies to become more available to the interaction of others on a global scale, where today's modern air, land and sea travel ensure rapid exposure to a greater mix and at a speed that has likely unheralded in our evolutionary past. These interests are far from academic since the practical considerations to human and animal health can have wide reaching implications for new methods of interdiction to combat evolving and newly emerging threats.

Baltic COMPASS and Biosecurity

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The EU project Baltic COMPASS is the result of a large number of international projects in different areas such as land-use, agriculture, water and environmental tasks, all related to the protection of the Baltic Sea. Baltic COMPASS is based on six WPs:

1. Management and Administration.
2. Communication and Information.
3. Best Practice Utilization and Transfer.
4. Environment Investment Preparation.
5. Comprehensive Assessments and Scenarios
6. Governance and Policy Adaptation

This presentation will focus on a specific objective in Baltic COMPASS and other projects, which are linked to biosecurity (WP5) in relation to intensive agriculture and animal production, especially zoonotic diseases. Knowledge on the fate, transmission and survival of zoonotic pathogens from different potential points sources such as sewage treatment works, animal farms and agricultural areas is very limited. Also, the use of antibiotics and the practice of land application of animal waste on environmental quality and health risks are not clear and there are many questions to answer. In addition, the complex relation and interactions between pathogens, pesticides, antibiotics, abiotic conditions, biological mechanisms and environmental health are challenges to investigate and significant future issues, which will be discussed.

Echinococcus multilocularis in rodents - when size matters?

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The fox tape worm *Echinococcus multilocularis* (EM) was recently detected in Sweden for the first time. The route of parasite transmission involves fox, or mainly other members of the Canidae, as the main host and rodents as indispensable intermediate hosts. Whilst the main hosts of Canidae experience little or no harm from the adult parasite tape worm, the larval form develops hydatid cysts that may severely affect fitness of the intermediate hosts'. Humans may, following accidental spill-over infection of the parasite's eggs, develop alveolar echinococcosis. In the literature of mainland Europe, there is an obvious skew regarding *Arvicolinae* rodent species afflicted as intermediate hosts. This raises the question whether competence as intermediate host varies between different *Arvicolinae* rodent species, or if one or several other influential factors are at play?

In pursuit of the EM parasite among Swedish rodents, and in line with the investigation of developing a feasible surveillance system, local rodent populations were sampled in the vicinity of the first location of EM discovery. The aim of the expedition was to maximise amount of "fox forage" sampled among EM competent intermediate hosts in relation to effort, thus no particular statistical design was applied except for the "hit & run", i.e. distributing as many sampling units per labour effort.

More than 200 rodents were sampled during April 2011 of the species *Arvicola amphibius* (former *A. terrestris*), *Microtus agrestis*, *Myodes glareolus*, *Apodemus sylvaticus*, and *Apodemus flavicollis*. Body size and patch abundance vary between the species, with *A. amphibious* being the largest in size and within small scale patches likely exhibiting the highest biomass.

Since predators make behavioural decisions on foraging, i.e. "optimal foraging", this lead to the increased tendency to forage in a non-random way to maximize energy intake. Predators may be attracted from long distances to localized patches of e.g. high prey biomass density, here the rodent species that represent the highest rate of biomass captured per effort.

Within a rational surveillance of EM by use of rodent sampling, in Sweden or elsewhere, it may be the best cost-efficient strategy to focus on the species of high local densities and thus probability of attracting fox. In such favourable patches, the probabaility of foxes defecating, and thus deposit EM eggs, may also be considerably higher than in other areas within the respectively foxes' home ranges. This may in turn render these rodent populations higher probability of EM egg exposure from fox feces, and subsequent higher prevalence to EM infection.

Microevolution of Puumala Hantavirus throughout a bank vole population dynamics

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Puumala hantavirus (PUUV) is the causative agent of Nephropathia epidemica (NE), a mild form of hemorrhagic fever with renal syndrome (HFRS). NE is a zoonosis occurring throughout Europe. More than 10 000 cases of NE are annually diagnosed in Europe. Our goal is to gain insights into microevolution of PUUV in relation the bank vole population dynamics. We are monitoring PUUV genomes circulating in natural foci of infection. Inspection of the wild-type PUUV populations revealed the following:
- a great genetic diversity among PUUV variants;
- the genetic diversity of the L genome segment, encoding for the RNA-polymerase, is two-fold higher than those of the S and M segments encoding structural components of the virus;
- reassortment occurs frequently (15-30%), and follows a certain pattern;
- PUUV variants belonging to different genogroups of the same lineage, or even distinct lineages, can co-circulate and interact with each other within a given bank vole population;
- the observed newly generated reassortant variants over the bank vole population cycles are less than the parental variants; this suggest a lower fitness of the reassortants compared to the parental variants;
- the frequency of recombination in PUUV is much modest that the reassortment frequency. No recombinant variants survived from one season to the next.
This study is ongoing and we believe that further details of PUUV microevolution in relation to the population dynamics of its host will be ascertain in the nearest future.

Screening for potential interacting partners of hantaviral non-structural proteins

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Hantaviruses (family Bunyaviridae) are single-stranded RNA viruses with tripartite genome. Several of them are considered to be emerging zoonoses. Unlike other members of the family, which are spread by arthropods, their native hosts are rodents such as mice and voles. Each virus species is usually associated with particular rodent host causing persistent and most likely asymptomatic infection. Although human infection is always incidental, hantaviruses can cause serious, life-threatening diseases such as hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS). In Finland alone there was over 1400 cases of nephropathia epidemica, a milder form of HFRS in 2010. Hantaviral genome consists of three proteins: polymerase for RNA replication, multifunctional nucelocapsid protein which forms the viral shell and glycoprotein which binds to receptors on the host cell surface. Additionally some of the viruses produce non-structural (NS) protein(s) using alternate reading frame strategies. The discovery of these NS proteins is fairly recent, and their function(s) are not yet understood. In a recent study we used yeast two-hybrid screening for finding potential binding partners of two related NS proteins and produced an interactome of possible NS protein partners. This data was then used to select promising candidates for further biochemical analysis.

(Molecular) epidemiology, pathogenesis and diagnostics of Sindbis virus infection

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Arthropod-borne Sindbis virus (SINV) is a enveloped single-stranded RNA virus of the genus Alphavirus in the family Togaviridae. SINV is found in Eurasia, Africa and Oceania but clinical infections are reported almost exclusively from Northern Europe, particularly from Finland where clinical SINV infection is called Pogosta disease. Acute SINV infection is characterized by fever, rash, myalgia and arthritis. Long-term joint manifestations can persist even for years.

SINV epidemics have a peculiar cyclic appearance in Finland and during large outbreaks several hundreds of cases are reported (Sane et al., 2010). Population density fluctuations of grouse, considered probable natural hosts for the virus, and alterations in climatic and weather conditions, may be involved in the observed epidemiological pattern.

Our research aims include characterisation of epidemiological risk factors of SINV infection and modelling of epidemics, molecular epidemiology of Finnish SINV strains, and development of diagnostics as well as elucidating the pathogenesis of SINV arthopathy and myalgia and association to onset of autoimmune diseases. All projects are carried out with extensive collaboration network.

We recently published a population based case-control study on risk factors of SINV infection (Sane et al.,2011) and are at the moment studying the association of environmental and weather factors with SINV epidemics using time series modelling. On SINV molecular epidemiology, we have sequenced the full length protein coding area of all human SINV isolates (isolated and partly sequenced earlier in our laboratory) as well as one novel strain recovered from mosquitoes and detailed phylogenetic analyses of these strains are ongoing. We have recently developed a one step real- time RT-PCR for detection of SINV and evaluated its clinical performance (Sane et al. submitted) with acute-phase serum samples.

Concerning pathogenesis, we have preliminary evidence that HLA alleles B*35 and DRB1*01 and complement component C4Q0 alleles are associated with SINV infection and our studies on muscle biopsy taken from a patient with chronic SINV infection together with in vitro studies on susceptibility of muscle cells for SINV, have provided new insights into SINV pathogenesis.

Complement activation in Puumala hantavirus infection correlates with disease severity

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Hantaviruses are important human pathogens that cause clinical diseases characterized by renal and cardiopulmonary manifestations. Their pathogenesis is currently poorly understood. A previous study on complement activation in PUUV infection showed that complement activation is common and suggested that the classical pathway of complement is associated with disease severity. However, the role of the complement system in the pathogenesis of PUUV-HFRS has not been further studied in larger patient populations.

We studied the activation of complement by measuring the terminal complement complex SC5b-9 and complement component C3 and C4 levels in patients with acute PUUV infection. Several laboratory parameters and clinical findings reflecting the severity of PUUV-HFRS were evaluated with regard to complement activation.

The levels of SC5b-9 were significantly increased and C3 decreased in the acute stage as compared to the levels at full recovery ($P<0.001$). We found that SC5b-9 levels were higher in patients with chest x-ray abnormalities than in patients with a normal x-ray during the acute stage ($P=0.028$). Furthermore, SC5b-9 and C3 levels showed significant correlation with several clinical and laboratory parameters that reflect the severity of the acute PUUV infection.

We showed that complement system becomes activated via the alternative pathway in the acute stage of PUUV infection and the level of activation correlates with disease severity. The results further suggest that complement activation may contribute to the pathogenesis of acute PUUV infection.

Isolation and Characterization of novel insectivore-borne hantaviruses and their pathogenic potential

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Hantaviruses are enveloped RNA viruses belonging to the *Bunyaviridae* family. Hantaviruses are globally important human pathogens causing two different syndromes: hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HPS). Both HFRS and HPS are acute febrile infections that differ in clinical manifestations: HFRS causes kidney failure, while the typical symptoms of HPS are pneumonia and cardiovascular dysfunction. Until recently, rodents have been regarded as the primary reservoir hosts of hantaviruses. However, during the last couple of years, novel hantaviruses have been discovered frequently from insectivore hosts.

The aim of the project has been to screen insectivore species for novel hantaviruses. Tissue samples of potential host species have been examined using RT-PCR. The objective is to isolate and characterize these newly detected hantaviruses, and to define their potential infectivity and pathogenicity to humans. The characterization will include the determination of basic features of these viruses in terms of growth kinetics, life cycle in cells, structure and function of proteins, and cell tropism in tissue culture. The first step in the characterization of these newly isolated viruses will be the production of antibodies either against the whole virus or a recombinant N-protein. The pathogenic potential will be studied by serological screening of human samples.

For now, three novel hantavirus species have been detected and isolated from Finnish shrews, and further studies are being conducted using these particular viruses called Asikkala, Laihia and Seewis

Tackling Climate Change: ECDC's Action Plan

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The predicament of climate change calls for concerted public health action. In Europe the incidence, prevalence, and distribution of vector-, rodent-, water-, and food-borne infections are expected to shift in a changing environment. Due to the high level of uncertainty on the rate and speed of climate change and its impact on infectious diseases, the European Centre for Disease Prevention and Control (ECDC), a new public health agency in Europe, has mounted a proactive public health response.

ECDC has developed a climate change knowledge-base for food and waterborne diseases in order to assess the evidence-base for climatic and environmental factors. This climate change knowledge-base has revealed a rich web of interconnections between specific pathogens and climatic and environmental variables. The knowledge-base can help to disentangle some of these complex relationships and help elucidate the impact of climate change on food and waterborne diseases.

ECDC has also completed a number of projects such as an assessment of the current magnitude and importance of vector-borne diseases in a changing European environment and a map that shows the precise current distribution of *Aedes albopictus*, the vector of chikungunya and Dengue with its climatic range.

ECDC is currently in the process of building an integrated network for environmental and epidemiological data. The blueprint of such a European Environment and Epidemiology (E3) Network has been designed and integrated into the IT landscape of ECDC. It will be connected with the mandatory surveillance system of 49 diseases and with epidemic intelligence that monitors threats and outbreaks in Europe and beyond. The E3 Network will have the capacity to connect epidemic intelligence and infectious disease surveillance with meteorological, entomological, water quality, remote sensing, or other data, for multivariate analyses and long-term projections. Merging, integrating, and analyzing such environmental and epidemiological data will advance our understanding of the relationship between climate change and infectious diseases in Europe and inform public health action.

ECDC has initiated and coordinated the development of a Handbook as an aid for EU Member States to assess and manage changes in risk of infectious diseases posed by climate change. The Handbook draws on current scientific knowledge as well as experiences and best practices from previous national risk, vulnerability, and adaptation assessments. The aim is not a complete review of the scientific field, but to give suggestions, tools, and hands-on approaches on how to access data, and choose organisational structure and analysis methods for a national assessment based on local conditions, competence, and aims.

With this action plan, ECDC hopes to be able to mount an effective response to reduce health impacts from climate change.

Genetic characterization of arenaviruses in small mammals

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Arenaviruses (family *Arenaviridae*) are enveloped negative-stranded RNA viruses that are associated with specific rodent reservoirs. In rodents, arenaviruses cause chronic infections, while in humans, they may cause hemorrhagic fevers. In Europe, the only arenavirus reported is lymphocytic choriomeningitis virus (LCMV), traditionally associated primarily with the common house mouse, *Mus musculus*. Humans infected with LCMV are usually asymptomatic or they show mild flu-like symptoms, but the infection may lead to aseptic meningitis, meningoencephalitis, and congenital abnormalities. LCMV or LCMV type viruses are circulating also among wild rodents, and several species around Europe have been found seropositive in EDEN studies. Attempts to amplify arenavirus genome sequences from the wild rodents have been mainly unsuccessful, and only recently an independent lineage of LCMV was described from wood mice (*Apodemus sylvaticus*) captured in Spain.

We have aimed to screen wild rodents for arenavirus genomes, and perform unbiased whole genome sequencing to recover full length genomes of arenaviruses from rodent tissue samples collected in EDEN.
As an example of our approach, wild rodents were trapped in Latvia and tested for arenavirus antibodies by using immunofluorescence assays (IFAs). From the study sites in which positive animals were found, 66 samples were chosen for screening by nested-RT-PCR targeting conserved motifs of the N gene using recently published primers (Ledesma et al., 2009). The collection of samples included both seropositive (32%) and seronegative rodents of the species *Myodes glareolus, Microtus arvalis* and *Apodemus* spp. LCMV-like genome sequences were recovered from three seronegative bank voles. This is the first time ever when arenavirus sequences have been recovered from *Arvicolinae*-rodents.

Monitoring MHC genetic diversity as a fast track to uncovering disease routes?

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When the environment changes the species inhabiting it need to adapt or other species will take over. For example, if the climate gets warmer so that the environment is suitable for a new vector-spread disease, the pathogen may spread through new vectors to new host populations. If the individuals in the host population cannot defend themselves against this new pathogen, the pathogen will be able to spread faster across the landscape and the fitness of the host population will decrease. For populations to adapt to new conditions, they need the raw material to do so. A population with high genetic diversity has a higher likelihood of holding a mutation that is suitable in the new circumstances than a population with low genetic diversity. Major Histocompatibility Complex (MHC) genes are the most diverse genes known in the vertebrate genome and are important in the immune defense. For example, MHC alleles have been identified to give resistance against certain pathogens and MHC diversity has also been linked to survival. Populations with higher MHC diversity may therefore cope better with new viruses or bacteria than populations with low MHC diversity. During my PhD, I have developed methods to characterize and genotype MHC genes in black grouse (*Tetrao tetrix*). Recently, I have demonstrated that small isolated black grouse populations have lower neutral genetic diversity and lower MHC class II B diversity than larger populations. Currently, I am testing whether MHC BLB1 and BLB2 heterozygosity are associated with fitness and if certain BLB alleles give pathogen resistance in black grouse. By monitoring MHC diversity in both wild and domestic host populations it may be possible to predict and identify populations were new diseases will spread faster.

New Opportunities for Terrestrial Research and Monitoring in the Arctic

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A huge international research and monitoring effort is directed towards northern territories such as the Arctic, the Subarctic, and the northern boreal region. The main reason for this is the rapid effects of climate-change that effectively contrasts with yesterday's relative pristinity. Permafrost meltsaway at unprecedented rates (the big thaw), snow-cover dynamics are rapidly changing, and vegetation characteristics profoundly altered. One process that forces these alterations is a northward propagation of expectancies regarding climate variables such as temperature, precipitation, and cloudiness, another is an increasing variance that forces weather events into the extreme. As a result, habitats of the vectors and reservoirs that carry infectious pathogens change, with altered human and animal exposure to infectious disease as a result. Since many Northern societies depend on reindeer and sheep husbandry, hunting, fishing, and tourism, changing health statuses strike at the heart of societal economy and culture. Despite the obvious severity of the above scenario, the altering health status of animals, with all the associated effects, is not sufficiently covered by existing research and monitoring organisations. Many studies are being performed on the correlation from changing climate/weather expectancy and variance to vegetation and animal effects, and also further onwards to the effects on husbandry and economies. Climate-change effects on the processes related to human and animal health are far less assessed, and an organisation that addresses the necessary combination of disciplinary skills is largely lacking. In short, in-field research regarding the processes that connects climate change, via changing habitat characteristics, with health dynamics is required. INTERACT (International Network for Terrestrial Monitoring and Research in the Arctic) is a network under the auspices of SCANNET, a circumarctic network of approximately 35 terrestrial field bases throughout Northern nations such as Sweden, Finland, Scotland, Faeroe Islands, Iceland, Greenland, Norway, and Russia. The INTERACT is delegated the responsibility of acting as a funding agency of Polar research through the period 2011 – 2014, where approximately 4 MEUR will be supplied in order to offer increased access to SCANNET infrastructures (the INTERACT Transnational Access Program – see www.eu-interact.org). Via 7 subsequent calls, free access to research facilities and field sites, travel support, logistics support, and free access to site-specific information and data, may be provided for a maximum visit-length of 3 months. Priorities will be given to those that have not previously used the infrastructure, to those working at more than one location for generating comparative studies, and to early career scientists. The INTERACT Transnational Access Program hereby invites applications for in-field research regarding climate-change effects on health dynamics in Northern regions. Please see the INTERACT web-portal for information on the associated procedures and contacts.

New markers in pathogenesis and for severe course of Puumala virus infection

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This presentation will be based on several articles recently submitted for publication. In addition, salient features of lethal cases of PUUV infection will be reported. The collaborators are from Haartman Institute, University of Helsinki and from Tampere University Hospital.

Hantavirus glycoprotein Gn interacts with N protein and regulates the interferon response

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Hantaviruses have a tri-segmented negative-stranded RNA genome. The S segment encodes the nucleocapsid protein (N), M segment two glycoproteins, Gn and Gc, and the L segment the RNA polymerase. Gn and Gc are co-translationally cleaved from a precursor and targeted to the cis-Golgi compartment. The Gn glycoprotein consists of an external domain, a transmembrane domain and a C-terminal cytoplasmic domain. In addition, the S segment of some hantaviruses, including Tula and Puumala virus, have an open reading frame (ORF) encoding a nonstructural potein NSs that can function as a weak interferon antagonist.
We investigated the interaction of TULV N protein and the TULV Gn-CT. The Gn protein is located on the Golgi membrane and its interaction with N protein has been thought to determine the cargo of the hantaviral ribonucleoprotein which is an important step in virus assembly, but direct evidence has not been reported. We found that TULV Gn-CT fused with GST tag expressed in bacteria can pull-down the N protein expressed in mammalian cells; a mutagenesis assay was carried out, in which we found that the zinc finger motif in Gn-CT and RNA-binding motif in N protein are indispensable for the interaction.
The antiviral response is mainly mediated by alpha/beta interferon. Recently the glycoproteins of the pathogenic hantaviruses Sin Nombre and New York-1 viruses were reported to regulate cellular interferon. We found that Gn-CT can inhibit the induction of IFN activation through Toll-like receptor (TLR) and retinoic acid-inducible gene I-like RNA helicases (RLH) pathway and that the inhibition target lies at the level of TANK-binding kinase 1 (TBK-1)/ IκB kinase-ε (IKKε) complex and myeloid differentiation primary response gene (88) (MyD88) / interferon regulatory factor 7 (IRF-7) complex.

Bank vole brain and bornavirus: inside, out and reversed

Long title:

Intracerebral Borna Disease Virus Infection of Bank Voles Leading to Peripheral Spread and Reverse Transcription of Viral RNA

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Bornaviruses, which chronically infect many species, can cause severe neurological diseases in some animal species; their association with human neuropsychiatric disorders is, however, debatable. The epidemiology of Borna disease virus (BDV), as for other members of the family Bornaviridae, is largely unknown, although evidence exists for a reservoir in small mammals, for example bank voles (Myodes glareolus). In addition to the current exogenous infections and despite the fact that bornaviruses have an RNA genome, bornavirus sequences integrated into the genomes of several vertebrates millions of years ago. Our hypothesis is that the bank vole, a common wild rodent species in traditional BDV-endemic areas, can serve as a viral host; we therefore explored whether this species can be infected with BDV, and if so, how the virus spreads and whether viral RNA is transcribed into DNA in vivo.
We infected neonate bank voles intracerebrally with BDV and euthanized them 2 to 8 weeks post- infection. Specific Ig antibodies were detectable in 41%. Histological evaluation revealed no significant pathological alterations, but BDV RNA and antigen were detectable in all infected brains. Immunohistology demonstrated centrifugal spread throughout the nervous tissue, because viral antigen was widespread in peripheral nerves and ganglia, including the mediastinum, esophagus, and urinary bladder. This was associated with viral shedding in feces, of which 54% were BDV RNA- positive, and urine at 17%. BDV nucleocapsid gene DNA occurred in 66% of the infected voles, and, surprisingly, occasionally also phosphoprotein DNA. Thus, intracerebral BDV infection of bank vole led to systemic infection of the nervous tissue and viral excretion, as well as frequent reverse transcription of the BDV genome, enabling genomic integration. This first experimental bornavirus infection in wild mammals confirms the recent findings regarding bornavirus DNA, and suggests that bank voles are capable of bornavirus transmission.

Intensive forest management shaping small mammal communities – impact on PUUV hantavirus transmission

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Intensive management of boreal forests in Fennoscandia has lead to a mosaic-like landscape of different aged forests. As bank voles, the host of Puumala hantavirus (PUUV, the causative agent of nephropathia epidemica in humans), prefer mature forests, we investigated whether the fragmentation of old forests has impact on PUUV transmission in bank voles. In this four-year study, we trapped small mammals twice a year in four different forest age classes. We show that PUUV infected bank voles occur in all forest age classes, yet numbers of total and PUUV infected bank voles were higher in mature forests. We also demonstrate that besides host density, the dilution effect, i.e. the presence of non-host species plays a role in PUUV transmission in boreal forests, suggesting a higher human disease risk in mature forest that are characterised by high bank vole abundance and dominance. Both the proportion of a superior (*Microtus* sp.) and an inferior (*Sorex* sp.) competitor were inversely associated with PUUV infection probability in bank voles.

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